REMARKS

Claims 1, 4, 5, 8, 9 and 20, as herein amended, and claims 12, 14, 17, 18, 23, 25, 28, 29, 45 and 46, as previously presented are pending in the application. Claims 2, 3, 6-11, 13, 15, 16, 19, 21, 22, 24, 26, 27, 30-44, and 46-50 have been cancelled without prejudice or disclaimer. Applicant respectfully contends that the grounds of rejection set forth in the action with regard to the cancelled claims are rendered moot.

Claims 2, 5, 6, and 9 are objected-to for reciting certain claim informalities. Applicant has amended these claims to overcome the objections as follows. Claims 2 and 6 have been cancelled. Claims 5 and 9 have been amended to recite "said" elements according to the Examiner's helpful suggestions. Applicant respectfully requests that the Office withdraw these objections in view of his amendments.

Applicant notes that the Action contains the statement that the application names joint inventors. Applicant believes he is the only named inventor in this application, and requests clarification.

1. The claims as amended fulfill the requirements of 35 U.S.C. §112, first paragraph

Claims 2 and 6 stand rejected for failing to satisfy the enablement requirement of 35 U.S.C. §112, first paragraph. Without acquiescing to these grounds of rejection, and solely in an effort to expedite prosecution of the pending claims to allowance, Applicant has cancelled claims 2 and 6 without prejudice or disclaimer for filing in a related application.

Applicant respectfully contends that these amendments overcome the asserted grounds of rejection, and respectfully request the Examiner withdraw these grounds of rejection.

2. The claims as amended fulfill the requirements of 35 U.S.C. §112, second paragraph.

Claims 1, 2, 4-6, and 8 stand rejected under 35 U.S.C. §112, second paragraph as being indefinite. Applicant has amended these claims to overcome the grounds of rejection as follows.

Claim 1 has been amended to remove embodiments involving signal amplification, while claim 5 has been amended to recite embodiments wherein the primers or probes are detectably labeled. Applicant respectfully contends that these limitations would not be indefinite to the skilled worker, amend that this ground of rejection has been overcome.

Claims 2 and 6 are cancelled herein without prejudice or disclaimer, thus overcoming these

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grounds of rejection.

Claims 4 and 8 have been amended to recite that the detected RNA species is a tumor-related species, thus overcoming these grounds of rejection.

Applicant respectfully contends that these amendments overcome the asserted grounds of rejection, and respectfully request the Examiner withdraw these grounds of rejection.

3. <u>The claims are not rendered obvious by the cited prior art</u>.

Claims 1, 4, 5, 8, 9, 12, 20, 23, 45 and 46 are rejected under 35 U.S.C. §103 as being obvious over the teachings of Balazs in view of the teachings of Korneluk.

Claims 14 and 25 are separately rejected under 35 U.S.C. §103 as being obvious over the teachings of Balazs in view of the teachings of Korneluk.

Claims 17, 18, 28 and 29 are separately rejected under 35 U.S.C. §103 as being obvious over the teachings of Balazs in view of the teachings of Korneluk.

Applicant respectfully contends that their arguments traversing these separate grounds of rejection over the same prior art can be addressed most efficiently by making the distinctions between the claimed invention and the cited art in an integrated fashion, as follows.

The Supreme Court has set forth the methodology for determining obviousness for patent claims. First, the scope and content of the prior art must be assessed, and then compared with the claims taken as a whole. This analysis must be performed in view of the person of ordinary skill in the art, and requires a determination of the level of skill of the person of ordinary skill. Finally, any objective evidence of non-obviousness, like commercial success or the failure of others, must be considered. *Graham v. John Deere Inc.*. The burden is on the Office to assert a prima facie case of obviousness, and the Office must consider all rebuttal evidence of non-obviousness provided by Applicant. Finally, the Office cannot use an Applicant's own disclosure, the written description of Applicant's invention, or any of the prior art, to reconstruct the invention using hindsight. *KSR v. Teleflex Int'l.*

Applicant wishes to make clear that he does not dispute that experimental controls have been used in the diagnostic arts to reduce errors in detection. Applicant has not argued that the use of the controls recited in his claims is the distinction drawn with the references cited in this (and previous) Actions. Instead, Applicant respectfully contends that the Office has used hindsight to supply the information that a reference human group or population without disease can serve as a positive

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reference, i.e. could be reliably used as set forth in Applicant's claims. This is because prior to Applicant the art did not teach that extracellular RNA could be detected in individuals without disease in a form that could be used as a control as recited in the pending claims.

The Office combines the teachings of Balazs (which Applicant continues to contend do not teach as broadly as the Office has interpreted it) with the Korneluk reference, which is not specific for extracellular RNA but is understood to merely illustrate the use of control samples in assaying RNA expression (albeit intracellular RNA, as Applicant has argued previously). This *assumes* that the skilled worker would have expected that a reference sample from a human group or population without disease would be an appropriate reference sample, implicitly *assuming* that there would be extracellular RNA in the plasma or serum of said reference population to detect. The asserted rejection depends upon this assumption, since without it the skilled worker would have expected to detect nothing from the reference sample, a result that would have been experimentally indistinguishable from using water as the reference, and indistinguishable from a reaction that did not progress (due *inter alia* to failure to include a polymerase to an amplification reaction mixture). These results would be uninformative. Without the *assumption* made by the Office that the reference sample of plasma or serum from individuals without disease would contain detectable RNA species whose amplification would provide an appropriate control, the asserted obviousness rejection is without support.

The Office acknowledges that neither the Balasz nor Korneluk references contain this teaching; indeed, Applicant contends that the understanding in the art is precisely to the contrary. With regard to the Balazs reference, Applicant respectfully points out that the teachings of the Balazs reference are restricted completely to clinical cancer patients, as evidenced *inter alia* by these excerpts from the reference (as above, all citations are set forth herewith with reference to the translated text):

- "The invention concerns a method for detecting the specific mRNA sequence (target sequence) of substances <u>of cancer cell origin</u>…." (first sentence of spec.)

- "RNA transcripts or their specific fragments are released or deposited in detectable amounts and in a form protected against degrading enzymes <u>only by malignant cells</u>." (translated doc., page 7).

- "…after in vitro amplification, the malignancy test was positive only in the case of cancer, but not in the case of the precancerous adenomas." (translated doc., page 10).

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- "Because of the special properties of malignant cells and tumors, the method is suitable as a malignancy test…." (translated doc., page 12)

- "Claim 1. A malignancy test (cancer test) by in vitro enzymatic amplification of the specific RNA sequence of the substances of cancer cell origin…." (translated doc., underlined for emphasis)

These citations make clear that the Balazs reference affirmatively teaches that amplifiable RNA is *only* present in plasma when it is from cancer patients, due to the putatively "special properties" of cancer cells. There is no teaching in Balazs that amplifiable mammalian RNA can be detected in plasma from humans without cancer, or when the RNA is not of cancer cell origin. As such, Applicant respectfully contends that the pending claims cannot be considered obvious in view of the teachings of the Balazs reference, because the Balazs reference not only does not teach mammalian RNA from plasma or serum of a human without cancer, but actually teaches against it, explicitly stating plasma RNA is released "only by malignant cells" (page 7), even stating as example that plasma RNA could *not* be detected in those with premalignancy:

> Over 50% of adenomas with precancerous changes in the large intestine have ras oncogenes activated by point mutation (particularly Ki-ras), while cancerously changed adenomas have these oncogenes in a somewhat lower percentage. The mutation appeared most frequently with the Ki-ras oncogenes in the position of codon 12, and GGT is mutated to GAT in both conditions. The malignancy tests conducted without prior amplification of said mutated oncogene sequence from blood plasma RNA gave a negative result in both conditions, while after in vitro amplification, the malignancy test was positive only in the case of cancer, but not in the case of the precancerous adenomas. This example clearly shows that the invention, in spite of its increased sensitivity, is a reliable malignancy test. (p. 10)

Applicant respectfully contends that the Balazs reference thus contradicts any interpretation that its methods could be used to detect extracellular mRNA from blood plasma or serum from a human without cancer or other disease.

With regard to the understanding in the art (which is the only other source of support available to the Office, in view of the absence of support for the existence of amplifiable extracellular RNA in blood plasma, serum or other bodily fluid in a reference group or population without disease in either the Balazs or Korneluk references), Applicant respectfully contends that the evidence is contrary to the Office's position. As examples of this understanding, Applicant asks the Office to consider in this regard the Komeda reference (Komeda *et al.*, 1995, Cancer 75:2214-2219) and the Pfleiderer reference (Pfleiderer, 1995, Int. J. Cancer 64: 135-139), previously submitted.

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The Komeda reference teaches that "[i]t was *impossible* to detect free RNA extracted from Hep G2 cells when they were diluted once with control blood" (p.2216; emphasis added), using RNA isolation and RT-PCR amplification methods substantially similar to the methods taught in by Balazs. Pfleiderer, who also used RT-PCR amplification methods, teaches that the author "tested whether free . . . RNA in peripheral blood would also be detected . . .. As shown in figure 2 [of that reference], even high concentrations of . . . RNA (corresponding to 1% tumour cells) in peripheral blood were not detectable . . . indicated rapid and complete removal of free . . . RNA . . . by degradation . . ." (p.136). Further, the reference states that ". . . intact tumour cells are the *only* source of positive RT-PCR results. Free tumour RNA in blood, which might be released from cells . . . was not sufficiently stable to be detected . . ." (p. 137; *emphasis added*). Thus, the skilled worker would not have expected to be able to detect extracellular RNA in human blood plasma, serum or other bodily fluid, except for the teachings of the Balazs reference which, as discussed above and in Applicant's prior responses, were expressly limited to cancer patients due to purported disease-related idiosyncrasies thereof.

Moreover, as argued previously, the skilled worker would expect that even Balazs was able to detect RNA in blood plasma solely because he included RNase inhibitors, an aspect not required by Applicant's claims, and which confounds any positive teaching by Balazs. The issue is not whether Applicant's claims recite affirmatively that such RNase inhibitors are absent, but rather how the skilled worker would have interpreted the Balazs teachings. Because the art recognized that human blood plasma, serum or perhaps other bodily fluids provided a toxic environment for extracellular RNA, enriched in RNases that degrade extracellular RNA almost instantaneously, Applicant respectfully contends that the only reasonable expectation for the skilled worker is that Balazs had inadvertently detected intracellular RNA released as the result of the manipulations attendant upon extraction. Additional references support this conclusion, as follows.

Kamm et al. (Clinical Chemistry 18: 519-522, 1972), provide experimental data on the concentration of RNA in plasma when a ribonuclease inhibitor is mixed with whole blood prior to separation of plasma. Kamm et al report RNA concentrations in plasma of 144 mg/liter in the plasma of humans (abstract). One skilled in the art would recognize this plasma RNA concentration to be particularly high given the well established presence of nucleases in plasma, and would therefore conclude the findings reflect release of intracellular RNA during sample preparation. In contrast, subsequent art provides a better estimate of the concentration of *extracellular* RNA in

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plasma (i.e., as determined without mixing whole blood with a ribonuclease inhibitor prior to separation of plasma). Under such conditions, El-Hefnawy et al. (Clinical Chemistry, 50: 564-573, 2004) report results as follows:

> "Using real-time QRT-PCR, we verified the presence of amplifiable cell-free circulating RNA in every tested cell-free plasma sample from individuals without disease. The plasma RNA concentrations were in the range of 1-10 μg/L...." (page 567, top right hand column).

Experimental evidence as highlighted above thereby indicates that the concentration of circulating RNA in plasma is present in a range from 1-10 μg/L (El-Hefnawy et al). However, when whole blood is first mixed with an RNase inhibitor prior to the centrifugation process that isolates plasma (as performed by Kamm), consequent plasma RNA concentrations increase to a range about 144 mg/L, a greater than 10,000-fold increase in plasma RNA concentration. Experimental evidence thus suggests that the method of Kamm et al, and accordingly the method taught in the Balazs reference, results in intracellular RNA contamination of plasma comprising at least 99.993% of the RNA detected therein. In view of the high levels of intracellular RNA contaminant predicted using the Balazs method, taken in view of the Kamm reference, one of ordinary skill could have no expectation of success in amplifying *extracellular* RNA from plasma, nor does Balazs render the presence of amplifiable extracellular mammalian RNA obvious. Recognition of the presence of amplifiable extracellular mammalian RNA requires hindsight using the Applicant's disclosure, which is impermissible when asserting a prima facie case of obviousness. *KSR v. Teleflex Int'l., Id.*

Thus, neither the Balazs nor the Kamm reference would be understood by the skilled worker to suggest that extracellular RNA could be detected in blood plasma or serum, a disbelief consistent with the understanding in the art in the face of the Balazs reference as evidenced by the Komeda, Pfleiderer and Zhou references (also previously submitted). To the contrary, the skilled worker would recognize that the Balazs reference is inherently contradictory, or at best fails to provide all essential steps of a method, in that the reference instructs that one "does not generate RNA cell leakage", further "because this sensitive method can identify even small amounts of contamination", and yet despite this critical recognition Balazs fails to identify a manner in which cells can be separated without inducing such leakage, stating only "and the cells are removed." (Balazs, translation, page 12)

Applicant respectfully points out that even years after the Balazs and Kamm references, and following the instant invention, those skilled in the art still describe the presence of circulating

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extracellular RNA in plasma or serum as surprising:

- "The existence of circulating RNA is a *remarkable* finding because RNA is more labile than DNA and ribonuclease is known to be present in blood." (Tsui et al., Clinical Chemistry, 48: 1647-1653, 2002; page 1647, right hand column; *emphasis added*)
- "the possibility that extracellular RNA could survive in the blood was *not widely accepted* because plasma contains potent ribonucleases that should, in theory, destroy any free RNA." (El-Hefnawy et al., Clinical Chemistry 50: 564-573, 2004; page 564, right hand column; *emphasis added*).
- "A *recent* development in this field is the discovery of tumor-derived RNA in the plasma/serum of cancer patients. … The liability of RNA and the existence of ribonuclease in the plasma make it surprising that circulating RNA should be detectable at all." (Ng et al., Clinical Chemistry, 48: 1212-1217, 2002; page 1212, right hand column; *emphasis added*).

Generalized skepticism in the art supports a conclusion that Applicant's claimed invention was not obvious at the time the priority invention was made. "General skepticism in the art – not amounting to teaching away – is also relevant and persuasive evidence of non-obviousness. In effect, teaching away is a more pointed and probative form of skepticism expressed in the prior art." *Monarch Knitting Machinery Corp. v. Sulzer Morat GMBH*, 139 F.3d 877, 885 (Fed. Cir. 1998).

The Action asserts, in finding Applicant's previous iterations of this portion of the argument unpersuasive, that "since the selected RNase inhibitor does not cause any escape of RNA from the cell before of after their removal," RNA detected by Balazs must contain extracellular RNA species (Office Action at p. 18). Applicant respectfully contends that this argument improperly shifts the burden to Applicant, who has provided art-based evidence that the art (even after publication of the Balazs reference) believed that extracellular RNA was not present in blood plasma or serum. It is not that the RNase inhibitors cause the RNA to be released from blood cells – it is that the manipulations attendant upon isolating the RNA from blood plasma or serum release said intracellular RNA, which is stabilized by the RNase inhibitors added as taught by Balazs. The interpretation of the Balazs teaching in the Action is also not supported by the experimental evidence from the Kamm and El-Hefnawy references, which show that 99.993% of the RNA detectable in blood plasma is from intracellular RNA contamination, nor from the teaching of any other reference in the art.

Applicant also notes that the distinction the Action draws with the Zhou reference, that it illustrates experiments performed *in vitro*, and that "growth environments are different *in vivo* and *in vitro*." While not acquiescing to this interpretation, insofar as it constitutes a limitation in applying the teachings of the Zhou reference to rebut the asserted obviousness determination, it is equally

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disqualifying of the Korneluk reference (which also discloses the results of *in vitro* experiments) in support of the obviousness determination asserted in the Action.

Finally, with regard to the assertions in the Action that the rejection is based on the combination of Balazs and Korneluk, and that the Korneluk reference is not applied to remedy the deficiencies of the Balazs reference, Applicant will make his assertions another way. The skilled worker would have known from the combination of the Balazs and Korneluk references (interpreting the Balazs teachings according to the Action, which Applicant respectfully submits is an overbroad interpretation of those results that is inconsistent with the understanding of the art and indeed was rejected by the prior art as being unreliable) that RNA could be detected in blood plasma treated with RNases immediately upon blood draw, from cancer patients. Moreover, the skilled worker would have known that a reference sample was commonly used in the art to detect increased concentrations of RNA expression in cells *in vitro*, as taught by Korneluk. What the art did not teach, and what the Balazs reference affirmatively taught the skilled worker *not* to expect, was that a sample from a reference group or population comprising humans without disease was an appropriate, useful reference sample since RNA could be amplified and detected from the plasma of those without disease. While the prior art (such as the Kamm reference) describes detection of RNA in plasma from normal individuals, there is no prior art demonstrating that despite endogenous plasma nucleases such RNA maintains sufficient integrity as to be amplifiable and enable identification of specific RNA species. T o the contrary, it would be understood by one of ordinary skill in the art considering the totality of the teaching of the prior art (including the Komeda and Pfleiderer references) teach the opposite, that specific RNA species would not be identifiable from the plasma of those without cancer. Applicant's position is that this teaching is Applicant's alone and that the teaching is not found in the cited art. Accordingly, the use of Applicant's own teaching as the implicit basis for the asserted obviousness rejection is impermissible hindsight and the Office has thus failed to assert a *prima facie* obviousness rejection against these claims. *KSR v. Teleflex Int'l., Id.*

Applicant respectfully contends that the evidence presented herein establishes that the claimed invention is both surprising and unexpected, and that this evidence rebuts the asserted obviousness determination. Applicant thus respectfully requests that the Examiner withdraw these grounds of rejection.

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CONCLUSIONS

Applicant believes that all pending claims are in condition for allowance, and respectfully request that the pending claims be passed to issue.

If Examiner Lu believes it to be helpful, he is invited to contact the undersigned representative by telephone at (312) 913-0001.

Respectfully submitted,
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